

Detection of Specific Antibody in Adult Human Periodontitis Sera to Surface Antigens of *Bacteroides gingivalis*

Y. NAITO,* K. OKUDA, AND I. TAKAZOE

Department of Microbiology, Tokyo Dental College, 1-2-2, Masago, Chiba City, Japan

Received 10 July 1986/Accepted 10 October 1986

Sera from adult periodontitis patients were analyzed for antibody activity against surface antigens of *Bacteroides gingivalis* by immunoblotting analysis.

Bacteroides gingivalis has been recognized as causing adult periodontitis (13, 14). Antibody elevations in adult patients with periodontitis in response to the concentrations of the organisms have been reported by several authors (4, 6, 7, 15). In those studies, whole cells or crude extracts of the organisms were used to detect antibodies. The present study was done to determine which surface antigen was useful in detecting serum antibody to the organisms by immunoblotting analysis with monoclonal antibodies.

The surface antigens were extracted from *B. gingivalis* 381 cells grown in a diffusate broth as described previously (6). The extraction procedures and characteristics of lipopoly-

in each subject by the method of Ramfjord (9). Serum antibody levels were determined by a micro-enzyme-linked immunosorbent assay with alkaline phosphatase-conjugated goat anti-human immunoglobulin G (IgG) as described previously (6). In our preliminary experiments, the specific IgA levels against the antigens were lower than the IgG levels, and no significant differences were found between the IgM serum antibody levels in adults with periodontitis and those in normal adults. The antibody levels in the subjects are summarized in Table 1. No sera from normal individuals possessed high IgG levels to any antigen used. Almost all sera obtained from the patients reacted with sonicate anti-

TABLE 1. Reactivity of human antibodies with various antigens of *B. gingivalis* in the enzyme-linked immunosorbent assay

Serum sample	Age	Sex	% Bone loss ^{a,b}	Pocket depth (mm) ^{a,c}	Antibody level against:				
					Sonicate antigen	GESA	LPS	Capsule antigen	Hemagglutinin
Patients									
1	31	M	20	4.5	0.79	0.86	0.36	0.59	1.00
2	23	M	24	4.7	0.48	0.48	0.16	0.17	0.33
3	33	M	27	4.3	0.75	0.72	0.23	0.31	0.67
4	29	F	33	3.3	0.74	0.78	0.51	0.47	0.76
5	41	M	39	3.4	0.89	0.80	0.70	0.49	0.88
6	39	F	40	3.5	0.80	0.98	0.29	0.48	0.92
7	51	M	59	4.8	1.32	1.26	0.54	0.90	1.18
8	32	F	65	6.6	1.90	1.80	1.23	0.92	0.72
Mean ± SE			38.4 ± 5.7	4.4 ± 0.4	0.96 ± 0.15	0.96 ± 0.14	0.50 ± 0.12	0.54 ± 0.1	0.81 ± 0.1
Normal adults									
A	23	M	0	0.8	0.11	0.11	0.10	0.10	0.10
B	23	M	0	1.3	0.11	0.11	0.14	0.10	0.12
C	30	M	0	1.2	0.35	0.27	0.21	0.10	0.10
D	27	F	0	1.1	0.21	0.13	0.10	0.12	0.10
Mean ± SE			0 ± 0	1.1 ± 0.11	0.20 ± 0.06	0.16 ± 0.04	0.14 ± 0.03	0.11 ± 0.01	0.11 ± 0.01

^a Calculated by the method of Ramfjord (9) (see text).

^b See also Schei et al. (10).

^c See Ainamo et al. (1).

saccharide (LPS), capsule antigen, hemagglutinin, and glass bead-extracted antigen (GESA) were described previously (3, 4, 6, 8). Sera were collected from normal adults ($n = 4$) and adult periodontitis patients ($n = 8$). The normal adults had no deepened periodontal pockets and no detectable bone loss; the subjects with periodontitis had one or more periodontal pockets of at least 5 mm and bone loss detected with a radiogram (Table 1). Bone loss values represented average scores that were calculated on the basis of six teeth

gen, GESA, and hemagglutinin. Most sera from the patients reacted strongly with hemagglutinin, but a considerable variation in antibody levels against LPS and capsule antigen was found. A correlation analysis with Kendall TAU (12) was performed to determine the association between clinical indices and IgG levels against specific antigens. A statistically positive correlation was found between the IgG levels against the antigens and the degree of alveolar bone loss. On the other hand, no significant correlation was found between the antibody levels against the antigens and pocket depth. This result disagrees with the report of Schenck (11). Further

* Corresponding author.

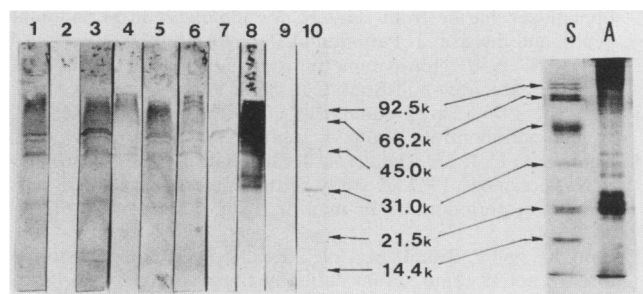


FIG. 1. Immunoblotting analysis of eight serum samples from patients with periodontitis and of one serum sample from a normal adult with GESA of *B. gingivalis*. Lanes: 1 to 8, serum samples from periodontitis patients 1 to 8; 9, serum sample C from a normal adult; 10, molecular weight markers (Bio-Rad Laboratories, Richmond, Calif.); S, sodium dodecyl sulfate-polyacrylamide gel electrophoresis pattern of molecular weight markers; A, sodium dodecyl sulfate-polyacrylamide gel electrophoresis pattern of *B. gingivalis* GESA stained with silver stain. Numbers in the middle represent molecular weights (in thousands [k]).

longitudinal studies of the antibody level changes in many patients are needed to clarify the role of specific antigens in the infectious process.

For immunoblotting analysis, GESA of *B. gingivalis* was mainly used. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (2) was performed with 12.5% slab gels, and immunoblotting analysis was carried out as described previously (5). After transfer, nitrocellulose strips were incubated with each serum sample diluted to 1:20 or 1:100 and probed with a 10^{-3} dilution of peroxidase-conjugated goat anti-human IgG (Cooper Biomedical, Inc., West Chester, Pa.). No binding of peroxidase-conjugated anti-human IgG alone to GESA was observed. Sera from almost all patients reacted clearly with GESA on the nitrocellulose paper. Sera from healthy individuals diluted to even 1:20 did not react with the antigen. Figure 1 shows the antibody binding patterns of sera obtained from patients with periodontitis. The numbers in Fig. 1 correspond to the serum samples in Table 1. The reaction patterns of serum samples 1, 3, 4, 5, and 6 were similar, but the strength of the reactions against the antigens varied from serum sample to serum sample (Fig. 1). The five serum samples clearly reacted with antigens with molecular weights of 57,000 and 43,000 and reacted as a minor band with antigens with molecular weights between 35,000 and 60,000. The 57,000-molecular-weight band formed with serum samples 3 and 5 was more distinct than any of the other bands. The antibody activity of serum sample 2, which possessed low IgG levels in the enzyme-linked immunosorbent assay, was notably weak. Serum sample 7 reacted specifically with an antigen with a molecular weight of 57,000. Serum sample 8 strongly reacted with the antigens as broad bands. The prominent reactions of serum sample 8 were with antigens with molecular weights of 57,000, 43,000, 38,000, and 35,000. After being washed with only phosphate-buffered saline instead of phosphate-buffered saline-Tween 20 solution, serum sample 8 showed regularly repeating bands throughout the length of the strip (data not shown). The pattern with serum sample 5 was quite similar to that with serum sample 8.

To identify the antibody to which the antigen reacted, inhibition assays were performed by the immunoblot method with monoclonal antibodies. The three monoclonal antibodies used recognized epitopes of LPS, capsule antigen, and hemagglutinin (5). GESA on nitrocellulose strips which had

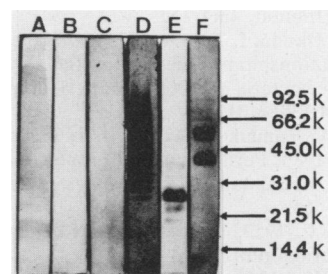


FIG. 2. Inhibition by pretreatment with serum sample 8 from a patient with periodontitis and serum sample C from a normal adult of monoclonal antibody binding of *B. gingivalis* GESA. The strips were pretreated with serum sample 8 (lanes A, B, and C) and with serum sample C (lanes D, E, and F) overnight. The washed strips were incubated with monoclonal antibodies A1.11 against LPS (lanes A and D), A3.81 against capsule antigen (lanes B and E), and C1.17 against hemagglutinin (lanes C and F). The strips were probed with peroxidase-conjugated goat anti-mouse immunoglobulins. Numbers at the right represent molecular weights (in thousands [k]).

been transferred from sodium dodecyl sulfate-polyacrylamide gels were incubated at 4°C overnight with serum from either a patient or a normal adult. After being incubated and washed, the strips were treated with the monoclonal antibodies at room temperature for 1 h. The strips were then probed with peroxidase-conjugated goat antimouse immunoglobulins. Figure 2 shows the typical patterns resulting from pretreatment with each sample of patient serum 8 and normal adult serum C. Pretreatment with serum sample 8 blocked the binding of monoclonal antibodies to capsule antigen and hemagglutinin and weakly blocked the binding of monoclonal antibody to LPS (Fig. 2). The blocking antibodies in serum samples from other patients were weak compared with those in serum sample 8. Pretreatment with sera from normal adults did not block any binding. The results indicate that the sera from patients with periodontitis recognized some antigenic determinants of the organisms.

It is interesting that most sera from patients with adult periodontitis possessed high antibody levels to hemagglutinin. This result indicates that adhesive surface structures such as hemagglutinin participate as a first step in *B. gingivalis* colonization and antigenic stimulation. The present findings emphasize the use of hemagglutinin to detect antibodies against *B. gingivalis* during the initial stages of adult periodontitis.

LITERATURE CITED

1. Ainamo, J. 1978. Assessment of periodontal status and treatment needs. W.H.O. Tech. Rep. Ser. 1978:28-43.
2. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* 227:680-685.
3. Mashimo, J., M. Yoshida, K. Ikeuchi, S. Hata, N. Kasai, K. Okuda, and I. Takazoe. 1985. Fatty acid composition and Shwartzman activity of lipopolysaccharide from oral bacteria. *Microbiol. Immunol.* 29:395-403.
4. Mouton, C., P. Hammond, J. Slots, and R. J. Genco. 1981. Serum antibodies to oral *Bacteroides asaccharolyticus* (*Bacteroides gingivalis*): relationship to age and periodontal disease. *Infect. Immun.* 31:182-192.
5. Naito, Y., K. Okuda, T. Kato, and I. Takazoe. 1985. Monoclonal antibodies against surface antigens of *Bacteroides gingivalis*. *Infect. Immun.* 50:231-235.
6. Naito, Y., K. Okuda, and I. Takazoe. 1984. Immunoglobulin G response to subgingival gram-negative bacteria in human sub-

- jects. Infect. Immun. 45:47-51.
7. Naito, Y., K. Okuda, I. Takazoe, H. Watanabe, and I. Ishikawa. 1985. The relationship between serum IgG levels to subgingival gram-negative bacteria and degree of periodontal destruction. J. Dent. Res. 64:1306-1310.
 8. Okuda, K., A. Yamamoto, Y. Naito, I. Takazoe, J. Slots, and R. J. Genco. 1986. Purification and properties of hemagglutinin from culture supernatant of *Bacteroides gingivalis*. Infect. Immun. 54:659-665.
 9. Ramfjord, S. P. 1959. Indices for prevalence and periodontal disease. J. Periodontol. 30:51-59.
 10. Schei, O., J. Waerhang, A. Lovdal, and A. Arno. 1959. Alveolar bone loss as related to oral hygiene and age. J. Periodontol. 30:7-15.
 11. Schenck, K. 1985. IgG, IgA and IgM serum antibodies against lipopolysaccharide from *Bacteroides gingivalis* in periodontal health and disease. J. Periodontal Res. 20:368-377.
 12. Siegel, S. 1956. Non-parametric statistics for the behavioral sciences. McGraw-Hill Book Co., New York.
 13. Slots, J. 1977. The predominant cultivable microflora of advanced periodontitis. Scand. J. Dent. Res. 85:114-121.
 14. Tanner, A. C. R., C. Haffer, G. T. Bratthall, R. A. Visconti, and S. S. Socransky. 1979. A study of the bacteria associated with advancing periodontitis in man. J. Clin. Periodontol. 6:278-307.
 15. Tolo, K., and P. Brandtzaeg. 1982. Relation between periodontal disease activity and serum antibody titers to oral bacteria, p. 270-282. In R. J. Genco and S. E. Mergenhagen (ed.), Host-parasite interactions in periodontal diseases. American Society for Microbiology, Washington, D.C.